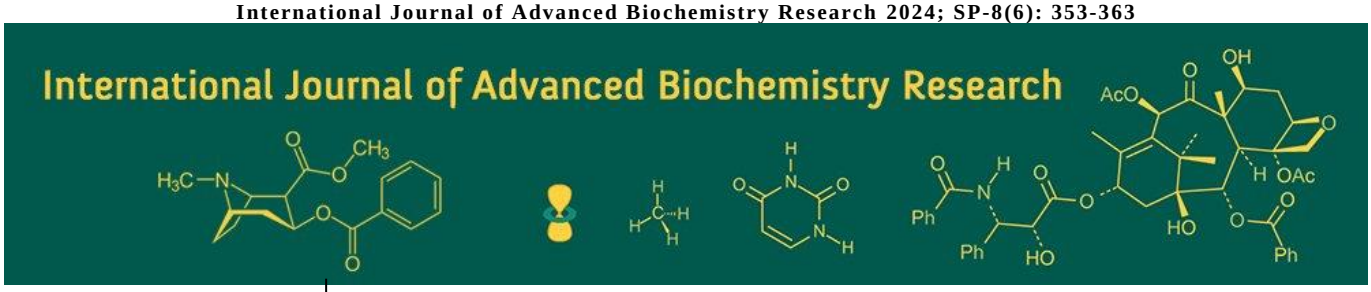

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Suppression of basal stem rot (*Sclerotium rolfsii*) of tomato by biofortified vermicompost

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Abstract
Tomato (*Solanum lycopersicum*) is a broadly developed and flexible natural product. Tomato generation faces different imperatives, both biotic and abiotic stresses. Fusarium shrink, caused by the soil-borne organism Sclerotium rolfsii a destroying malady that influences tomato plants around the world, causing noteworthy financial misfortunes in tomato development. In this ponder the potential utilize of biofortified vermicompost for shrivel administration was examined. The organic control specialists *viz*. *Trichoderma harzianum*, Pseudomonas fluorescens and Bacillus subtilis were utilized to braced the vermicompost. The ponder checked different cancer prevention agents, plant development markers, and malady rates over assigned medicines at diverse interims. The discoveries uncovered considerable contrasts: tomato plants treated with biofortified vermicompost appeared striking decreases in malady event, moved forward development, expanded yields, and increased antioxidant movement.

Keywords: Suppression, stem, *Sclerotium rolfsii*, vermicompost, *Solanum lycopersicum* L.

Introduction
Globally, tomatoes (*Solanum lycopersicum* L.) are one of the most extensively cultivated vegetables. Vegetables are a valuable food source for humans because they include a number of vital nutrients, including vitamins, carbs, and amino acids. Tomato and tomato products are rich source of vitamin C, potassium, folate. Tomato is regarded as the most sensitive vegetable species to excessive soil moisture (Iden, 1956) [1]. To feed the expanding population, there is a daily increase in the need for food. However, agronomic practices play a major role in food production. The main issues in food production are biotic and abiotic stresses, which lower productivity. The three primary abiotic variables that can have an impact, either directly or indirectly, are temperature, humidity, and soil properties. These abiotic variables can influence not only the direct impact on development but also the proliferation and damage caused by pests and disease. The biotic stresses which are most devastating and caused huge economic losses are collar rot, stem rot, damping-off, black rot fungal and bacterial wilt.

Sclerotium rolfsii occurs in soil as a saprotroph and attack living plants. Sclerotium rolfsii is a soil-borne fungus, facultative parasite and Omni pathogenic organism which occurs worldwide and infects more than 500 plant species including tomato, cucumber, maize, bean, soybeans, Brinjal, watermelon etc. It causes different types of diseases such as collar rot, sclerotium wilt, stem rot, charcoal rot, seedling blight, damping-off, foot-rot, stem blight and root-rot in many economically valuable crops. It was first observed by Peter Henry Rolfs in the year 1892 on tomato plants with 70% losses. The hyphae grew upward on the surface of the infected plant covered with a cottony, white mass of mycelium, scattered inside and outside of infected stem nearby the soil surface. The fungus produced numerous small round, white sclerotia of uniform size when immature and dark brown at mature stage.

Vermicompost is produced when various organic materials are broken down in a non-thermophilic way by earthworms and associated microbes. This breakdown produces a particularly fertile material with high porosity, aeration, and low C:N ratios.(Dominguez and Edwards, 2011) [2]. The notable characteristics of vermicompost that encourage plant growth are ascribed to the nutrients it contains, which include nitrates, exchangeable calcium, phosphorus, and soluble potassium in an available. (Gupta *et al*., 2014) [3].

In addition to improving soil biodiversity by significantly increasing microbial biomass, the application of compost and vermicompost as soil fertilizers aids in maintaining and rebuilding soil fertility (Kumar *et al*., 2013; Passarini *et al*., 2014) [4]. Applying biological control, such as vermicompost and vermicompost tea, is essential as a secure and effective substitute against these illnesses. In recent years, biological control has been recognized as crucial for the management of numerous soil-born pathogens. (Abada and Ahmed 2014; Abada and Hassan 2017; Ragabmana *et al*., 2015; Devi and Das 2016) [5].

Materials and Methods

2023–2024 saw little progress on the current study, "Development of potential Trichoderma based formation for management of collar rot disease of tomato caused by sclerotium rolfsii." The following is a description of the specifics of the materials and methods used throughout the inquiry.

Experimental site: The *in vitro* studies were carried out at the Institute of Agricultural Sciences, Ram University, Kanpur, India, at the plant health clinic and biocontrol laboratory of the department of mycology and plant pathology. The same department's polyhouse and agricultural field served as the sites for the *in vivo* studies. There, tomato crops were grown in earthen pots measuring 20 by 10 cm, with every physical safety measure taken to shield the plants from outside danger. Throughout the experiment, the experiment siteremained unchanged. The university is situated in an Indian state in the north. This town is 80 km west of Lucknow, the state capital, and is situated on the Ganga's south bank.

Collection of diseased samples

Random roving method of survey was carried out to record the severity of Sclerotium rolfsii in tomato. The survey was conducted during Rabi season 2023 to 2024.in 7districts of Eastern Uttar Pradesh i.e. Hamirpur, Mahoba, Banda, Jalun and Jhansi Chitrakoot, Lalitpur (Figure 1). The disease severity were estimated on the basis of individual plants scoring on a 0-5 visual scale (3.1) of increasing severity (Latunde-Dada 1993) [6]. While the mean disease rating (MDR) was calculated according.

Isolation of the pathogen

Isolated of pathogen on PDA medium from infected root of tomato plant. A small portion of diseased tissue along with a portion of adjacent healthy tissue were cut into small pieces (4 to 5 mm in length) and then surface sterilized with 0.1% HgCl2 for 30 sec. After that the pieces rinsed thrice with sterilized distilled water. Sterilized and rinsed pieces were inoculated aseptically on sterilized Petriplates which containing PDA (Potato dextrose agar) medium. The inoculated Petriplates were incubated at 20-25 ºC for 4-6 days. When grown the fungal colony, a small cut was made on mycelium with the help of cork borer and was transferred on another Petriplates containing PDA medium to obtain pure culture. The mycelial bit was also transferred to fresh PDA slants in order to store it for future uses.

Maintenance and storing of the pathogen

The pure culture of the pathogen Sclerotium rolfsii was maintained on PDA slants throughout the period of investigation by periodic sub culturing on fresh media and stored in a refrigerator at 4 ºC.

Pathogenicity test

The tomato cultivar "Kashi Anupam" was used for testing the pathogenicity of the pathogen. Tomato seeds were surface sterilized by using 1% sodium hypochlorite for 30 sec and was rinsed twice with sterilized distilled water and then air-dried. Soil mixture containing sandy loam soil, vermicompost, and farmyard manure (2:1:1) was autoclaved for 30 min at 15 lbs pressure for three consecutive days. Half amount of soil was also mixed with crushed mycelial powder of Sclerotium rolfsii. The seeds were sown inside 10 cm2 pots under greenhouse conditions. Untreated seeds sown in pathogen infected and pathogen uninfected soil served as positive and negative controls, respectively.

Preparation of Vermicompost

Temple, farmyard and kitchen wastes is used as feedstock in the present study. The temple wastes mainly consisted of Aegle marmelos leaves, Datura stramonium, *Tageteserecta* and Hibiscus *rosasinensis* flowers. The kitchen wastes were collected from the cafeteria of hostels in the Rama university campus and the remainder consisted of used tea leaves, flour, rice, pasta, bread, noodles, cooked vegetables and potatoes. Flesh, bones, fat, eggshells, etc. Were not included in the kitchen waste as they are not easily degradable and can be toxic to earthworms. The yard wastes mainly consisted of dried deciduous leaves (a mixture of *Mangifera indica*, *Sarakaasoka*, *Syzygium cumini*, *Tamarindus indica* and various grasses). Nine opaque, rectangular plastic boxes with dimensions measuring 340 cm × 160 cm × 60 cm, were used for vermicomposting and 45 holes, each of diameter 0.65 cm, were drilled at the base of the container for allowing proper exchange of gases. The experiment was set up in a randomized complete block design with three replications of each type of feedstock. Each feed stocks were kept for 2–3 days and thoroughly mixed before placing in the plastic boxes to avoid clumping and compaction of the ph following addition of water in each of the three feedstock, mature cow dung was added at a ratio of about 1:7 to provide an instant source of food to the earthworms. Finally the boxes were covered with a layer of soil for decomposition. Adult clitellate worms, Eiseniafetida, ranging in length from 4 to 8 cm were added at the rate of 1.5 kg/m2 through the developed cracks after 15 days of partial decomposition of waste to prevent worms from the thermophilic reaction occurring during composting. The moisture content of the feedstock was adjusted to 70 ± 10% at the start of vermicomposting and maintained throughout the period of vermicomposting by periodic sprinkling of water. Watering was stopped when the VC was ready as indicated by uniform dark brown to black coloured granular structure. Three days later the compost along with worms was harvested and the worms were removed by sieving (<2 mm) (Singh *et al*., 2013) [7].

Microbial fortification of vermicompost

The three BCAs *viz*. T. Harzianum, P. Fluorescens and B. Subtilis used in this study were chosen because of their compatibility and ascertained ability to reduce the soil borne diseases in various crops (Singh *et al*., 2013) [7]. All these selected BCAs were used to fortify the vermicompost individually. 1L of 2 days old bacterial cultures grown in

NB with CFU count approximately 1.8×108 was thoroughly mixed with 25 kg of freshly prepared vermicompost in separate trays while 1L of 5 days old T. Harzianum culture grown in PDB with CFU count approximately 2.8×107 was used to fortify other separate vermicompost tray (25 kg each). Trays were kept under shade and covered with dark polythene sheet for 10 days for acclimatization of BCAs.

Biological management of stem rot of tomato using Bio fortified vermicompost

Source of BCAs used and viability test

The biological control agents used in this study *viz*. Trichoderma harzianum, Pseudomonas fluorescens and Bacillus subtilis were obtained from the culture repository of Plant Health Clinic Laboratory of the Department of Mycology and Plant Pathology Rama University Kanpur U.P.

***In vitro* efficacy of BCAs against pathogen**

The antagonistic ability of selected BCAs against the pathogen was studied *in vitro* following a dual culture assay as described by Verma *et al*. (2007) [8]. A 9 mm disc (plug) of 15 days old cultures of Sclerotium rolfsii were cut with a sharp cork borer from the growing edge of the culture plate. The cut block was placed on PDA medium 1 cm away from the edge of the plate. 9 mm disc of biocontrol agent namely T. Harzianum isolate was placed at opposite end of the Petri plate. PDA plates inoculated with the pathogen alone served as the control and incubated at 25±2 ºC.

Similarly, the *in vitro* antagonistic ability of the bacterial isolates was studied using a dual culture assay described by (Zhang YuBo, ZhuangWenYing) [9]. A 9 mm plug of the Sclerotium rolfsii was placed at the centre of a Petri plate containing PDA, then the test bacterial isolate was streaked 3 cm away from the fungal plug at both the sides towards edge of the plate by a loop loaded with 48 h old bacterial culture. The plates were incubated at 2 8± 2 ºC for 7 days and the inhibition zone was measured from the edge of mycelium to the bacterial streaks, when the control plates showed full growth (Moctezuma HEF, Belmont RM, Perez AJ, Juarez RN) [10]. Per cent inhibition over control was calculated as per the following formulae given by Whipps (1997) [11].

$$PI=(C-T)/C\times 100$$

Where, PI = per cent inhibition over control C = Growth of test pathogen with absence of antagonist (mm). T = Growth of test pathogen with antagonist (mm)

Experimental details

Tomato seeds were surface sterilized by using 1% sodium hypochlorite for 30 sec and was rinsed twice with sterilized distilled water and then air-dried.

T_1: Vermicompost +*Trichoderma harzianum*+ Pathogen
T_2: Vermicompost + *Bacillus subtilis* + Pathogen
T_3: Vermicompost + *Pseudomonas fluorescens*+ Pathogen
T_4: Vermicompost + Pathogen
T_5: Control (Only vermicompost)

Details of the layout plan

Experimental design: Randomized completely block design

1. Number of treatments: 5
2. Number of replications: 3
3. Date of sowing:
4. First year(S1): October 23, 2022
5. Second year (S2): October 30, 2023
6. Total number of plots:24 x 2 x 2
7. Spacing: Row to row: 30 cm
8. Spacing: Plot to Plot: 30 cm

Pot experiments

Plastic pots of 15 cm ×10 cm were used to conduct the plant growth promotion and antagonistic potentials of fortified vermicompost against Sclerotium rolfsii. Soil was autoclaved for 30 min at 15 psi for three consecutive days. Pots were filled with soil mixture containing sterile soil and microbially fortified vermicompost in the ratio of 1:1 (w/w) (1.5 kg pot-1). In the first three treatments, vermicompost was fortified individually with T. *Harzianum, B. Subtilis, and P. Fluorescens* cultures as described above. Fourth treatment contained only vermicompost (positive control), while the fifth treatment contained only soil (Negative control).

Pathogen inoculation

The spore suspension of inoculum was prepared by pouring 20 ml of sterile distilled water in each culture plate of 5-7 days old fungal mycelium and then gently scraped using spore harvester. The concentration of conidia was adjusted to 2-3×107 conidia ml-1 using haemocytometer.5 ml of prepared spore suspension was used to inoculate each seedling in all five treatments using soil drenching method as described by Patil *et al*. (2011) [12]. In the soil drenching method, 5 ml of fungal suspension (i.e. water containing conidia of the pathogen) was inoculated to each of the seedlings by drenching the soil around the root zone with the help of pipette. Before inoculation, the roots were slightly severed (wounded) by inserting a needle, 1cm away from the stem. Root severing was done to ensure pathogen penetration through roots. Observations were recorded on symptoms of stem rot for up to 5 weeks.

Observations recorded

Random sampling technique was adopted for recording the observations of various morpho-physiological characters after 30, 60 and 90 days after sowing (DAS). When recording the data on various traits, three plants of each treatment from each replication were chosen at random. Replication-wise averages of the plant data were obtained, and mean data was utilised for statistical analysis. A set of suggested procedures was used to grow a crop that was healthy.

Morphological parameters

1. Shoot length (cm)Using a metre scale, the main shoot of the plant was stretched 30 days after sowing, and the distance was measured in centimeters from the base of the plant to the tip of the main axis.
2. Length of roots (cm)Using a meter scale, the distance was measured in centimeters from the plant's base at ground level to the tip of its main root at 30 days following seeding.
3. Dry weight (g) after washing the plants in the tap water and softly wiped with using blotting paper, fresh weight was determined by using an electronic balance (Sartorius BT-224S) and the values were expressed in grams. After taking fresh weight, the plants were placed

to 100 °C pre-heated hot air oven for one hour. Then they were placed in an oven, maintained at 60±2 °Cfor drying purpose. The weight was measured regularly and expressed in grams.

Biochemical Analysis

Biochemical analysis for determination of different antioxidants and ROS (H_2O_2) in the leaves of tomato plants at different time intervals after pathogen inoculation was performed according to the method of Singh *et al*. (2013). The enzymatic assays namely phenylalanine ammonia-lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO), superoxide dismutase (SOD) and total phenol content (TPC) was performed after 0, 24, 48, 72 and 96 h pathogen inoculation as described by Jain *et al*. (2020) [13].

Superoxide dismutase (SOD) assay

SOD (EC 1.15.1.1) activity was assayed following the method of Fridovich (1974) by measuring the ability of enzyme extract from samples to inhibit photochemical reduction of nitroblue tetrazolium (NBT) chloride. Fresh leaves (0.1 g) from each of the treatments were homogenized in 2.0 ml of extraction buffer (0.1 mol l-1 phosphate buffer containing 0.5 mmol l -1 EDTA at pH 7.5) in a prechilled mortar and pestle. The homogenate was centrifuged at 15 000 g for 20 min at 4 ºC. The reaction mixture contained 200 mmol l-1 methionine, 2.25 $mmolL^{-1}$ NBT, 3 mmol-1 L EDTA, 100 mmol L-1 phosphate buffer (pH 7.8), 1.5 $molL^{-1}$ sodium carbonate and enzyme extract. The final volume was maintained to 3 ml. Reaction was started by adding 2 l $molL^{-1}$ riboflavin (0.4 ml), and the tubes were illuminated with two 15 W fluorescent lamps for 15 min. Reaction mixture without enzyme served as control. The reaction was terminated by putting the light off and keeping the tubes in dark until the absorbance was recorded at 560 nm. One unit of the SOD activity was defined as the amount of enzyme reducing the absorbance to 50% in comparison to control lacking enzyme.

Phenylalanine ammonia-lyase (PAL) assay

Leaf sample of 0.1 g from each of the treatments was homogenized in 2 ml of (0.1 mol l-1 sodium borate buffer (pH 7.0; 4 ºC) containing 1.4 m $molL^{-1}$ ß-mercaptoethanol and centrifuged at 16000 rpm at 4 ºC for 15 min. The supernatant was used as enzyme source. To the reaction mixture containing 0.2 ml of enzyme extract, 0.5ml of 0.2 $molL^{-1}$ borate buffer (pH 8.7) and 1.3 ml of water were added. The reaction was initiated by the addition of 1ml of 0.1 $molL^{-1}$ phenylalanine (pH-8.7) and incubated for 30 min at 32 ºC. The reaction was terminated by addition of 0.5 ml of trichloroacetic acid (TCA, 1 M). PAL (EC 4.1.3.5) activity was measured following the formation of trans-cinnamic acid at 290 nm as described by Brueske (1980) [14] and was expressed in terms of $\mu molL^{-1}$ TCA per g fresh weight (FW).

Total phenolic content (TPC) assay

The TPC was determined following the method of Zheng and Shetty (2000) [15]. Leaf tissue (0.1 g) was placed in 5 ml of 95% ethanol and kept at 0 ºC for 48 h. The samples were homogenized individually and centrifuged at 13000 rpm for 10 min. To 1 ml of the supernatant, 1 ml of 95% ethanol and 5 ml of sterile distilled water and 0.5ml of 50% Folin–Ciocalteau regent were added, and the content was mixed thoroughly. After 5 min, 1 ml of 5% sodium carbonate was added, the reaction mixture was allowed to stand for 1 h and the absorbance of the colour developed was recorded at 725 nm. Standard curves were prepared for each assay using various concentrations of gallic acid (GA; Sigma-Aldrich-27645) in 95% ethanol. Absorbance values were converted to mg GA equivalents (GAE) g-1 FW.

Polyphenol oxidase (PPO) assay

Leaf samples (0.1 g) were homogenized with 2 ml ice cold phosphate buffer (0.1 mol l-1, pH 6.5). The homogenate was centrifuged at 16000 rpm for 30 min at 4 ºC and the resulting supernatant thus obtained was used directly in the enzyme assay. The reaction mixture contained 0.4 ml catechol (1m mol L-1) in 3 ml sodium phosphate buffer (0.05 mol L-1; pH 6.5) and 0.4 ml enzyme extract. Reaction mixture containing only substrate served as control. Catechol was used as substrate for PPO (EC 1.14.18.1) and increase in absorbance was recorded at 405 nm by (Ana Winters) [16]. The linear portion of the activity curve was used to express PPO enzyme activity as change in O.D. min-1 g-1 FW.

Peroxidase (PO) assay

PO (EC 1.11.1.7) activity was assayed by the method of Hammerschmidt *et al*. (1982), [17] with slight modification. Leaf samples (0.1 g) were homogenized separately in 2 ml of 0.1 mol l-1 phosphate buffer (pH 7.0), at 4 ºC, centrifuged at 16000 x g at 4 ºC for 15 min and the supernatant was used as enzyme source. The reaction mixture consisted of 1.5 ml pyrogallol (0.05 $molL^{-1}$), 0.05 ml enzyme extract and 0.5 ml H2O2 (1% v/v). Reaction mixture without the enzyme served as control. The changes in the absorbance at 420 nm were recorded after 30s intervals for 3 min. The enzyme activity was expressed as change in the U min-1 g-1 FW.

Determination of Disease Incidence

The disease incidence was recorded on a scale of 0–4 referring to the degree of wilt as reported by Song *et al*. (2004) where scale zero refers to healthy plant without any wilt symptoms. On the other hand, scale four refers to complete wilted plants. The scale 1, 2 and 3 refers to different degrees of wilt which indicates the scale of disease severity. The scale 1- plant showed yellowing of leaves and wilting ranging from 1-20%; scale 2-plant showed yellowing leaves and wilting ranging from 21-40%; scale 3-plant showed yellowing leaves and wilting ranging from 41-60%. Scale 4- is when all leaves become yellow as an indication of complete infection. Disease incidence is a parameter which includes disease percentage and disease severity according to Song *et al*. (2004) [18] as given below:

$$\text{Disease incidence (\%)} = \frac{\sum \text{scale} \times \text{number of plants infected}}{\text{highest scale} \times \text{total number of plants}}$$

Results

Collection of diseased plant sample from different tomato growing districts of Eastern Uttar Pradesh (Bundelkhand region).

Table 1: Disease Severity rating

S. No.	Isolate name	Location	District	Disease Severity rating (1-9 rating scale)
1	Sor 1	Maudha	Hamirpur	8.2
2	Sor2	Kurarakvk	Hamirpur	5.1
3	Sor3	Sumerpur	Hamirpur	9.1
4	Sor4	BUAT	Banda	7.2
5	Sor5	Mawai	Banda	6.0
6	Sor6	Kabrai	Mahoba	6.2
7	Sor7	Nihalgarh	Chitrakoot	3.6
8	Sor 8	Kanpur	Kanpur	8.0
9	Sor 9	Daulatpur	Jalun	6.3
10	Sor 10	Datiya	Jhansi	5.8
11	Sor 11	Sumerpur	Sumerpur	7.3
12	Sor 12	Lalitpur	Lalitpur	7.1

Isolation, purification and maintenance of *Sclerotium rolfsii* Isolates

The Using the descriptions provided by C.M.I. (1970), the isolates were purified and identified as Sclerotium rolfsii based on morphological and cultural characteristics. From Sor 1 to Sor 12, the isolates were assigned sequential numbers. For later usage, the refined isolates were kept in PDA slants and refrigerated at 4 ºC.

Test of pathogenicity of *Sclerotium rolfsii* isolates

Following *Sclerotium rolfsii* extraction and purification from the collected samples, the pathogenicity of the tomato cultivar "Kashi Anupam" was tested using asusceptible genotype using the soil inoculation method. Six of the twelve Sclerotium rolfsii isolates that were examined for pathogenicity displayed stem, crown, or whole plant blight. On old lesions close to the soil's surface, white mycelium developed into sclerotia rolfsii after spreading along the stems of diseased plants. Disease symptoms including chlorosis, wilting, damping-off, blighting, necrosis, and ultimately plant death were displayed by the cultivars.

As a result, six isolates tested positive for Koch's postulate, while the other six isolates were unable to demonstrate Koch's postulate, proving they were not pathogenic to tomatoes. The six isolates that tested positive for Koch's postulate were chosen for additional investigation. Names for the chosen isolates included Sor 1, Sor 3, Sor 4, Sor 8, Sor 11, and Sor 12. These isolates' colony properties were investigated. The "soil inoculation" approach was used to further investigate the percent disease incidence (PDI) of these chosen isolates.

Table 2: Displays the pathogenicity

1	Sor1	+ve
2	Sor2	-ve
3	Sor3	+ve
4	Sor4	+ve
5	Sor5	-ve
6	Sor6	-ve
7	Sor7	-ve
8	Sor 8	+ve
9	Sor 9	-ve
10	Sor 10	-ve
11	Sor 11	+ve
12	Sor 12	+ve

Study of the effect of selected isolates of *Sclerotium rolfsii* on tomato in pots

Six distinct isolates of Sclerotium rolfsii were examined for their percent disease incidence (PDI) using soil inoculation techniques in pots kept in a greenhouse. Table 2 shows that the data were recorded between 30 and 120 DAI. Table 3 makes clear that none of the isolates had PDI up to 30 DAI, but three isolates-Sor 2, Sor 8, and Sor 12-recorded PDI at 60 DAI of 18.32%, 19.83% and 17.41%, respectively. At 90 DAI, the six isolates showed varying PDI levels. The highest PDI, or 34.20%, was seen during treatment with Sor3, and this was followed by Sor 8's 31.02% PDI. The values of PDI also dropped with the other isolates. Sor3 recorded the highest PDI amongst all isolate from 60-120 DAI while Sor 8 recorded the second highest position from 90-120 DAI. Four other isolates *i.e* or 1, Sor3, Sor8, and Sor 12 also recorded PDI more than 70.0% while the remaining ones i.e Sor4, Sor11 recorded PDI less than 70.0%.

Based on Table 3's results, it was determined that Sor3, a Hamirpur strain of Sclerotium rolfsii, was the most aggressive of the six isolates. As a result, it was chosen as the test pathogen for additional investigations.

Effect of selected *S. rolfsii* isolates on disease incidence of tomato through soil inoculation method**.** Results are expressed as mean of triplicates ± S.D.

Table 3: Percent disease incidence (%)

Name of the isolates	Percent disease incidence (%)				
	Days after inoculation				
	30	60	90	105	120
Control	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0
Fol1	0 ±0	0 ±0	21.63 ±5.25	47.75 ±8.22	70.29±13.21
Fol3	0 ±0	18.32±5.25	34.20±7.33	53.35 ±8.33	89.65±14.33
Fol4	0 ±0	0 ±0	23.74±6.38	42.12±9.75	65.95 ±14.33
Fol8	0 ±0	19.83±5.25	31.02±6.25	47.81±8.22	81.45±10.26
Fol11	0 ±0	0 ±0	21.40 ±8.45	31.92±9.25	62.76±12.63
Fol12	0 ±0	17.41±5.25	30.52 ±6.38	41.71±14.33	71.30±12.85

Biological management of collar rot of tomato by using biofortified Vermicompost

***In vitro* Efficacy of *BCAs* against Pathogens**

After four days, a dual culture test was used to assess the antagonistic activity of the previously characterized BCAs against Sclerotium rolfsii. The bio agents considerably decreased the radial growth of Sclerotium rolfsii, as Table 4 demonstrates. In comparison to T. harzianum and B. subtilis, P. fluorescens exhibited more antagonistic activity against the radial growth of Sclerotium rolfsii.

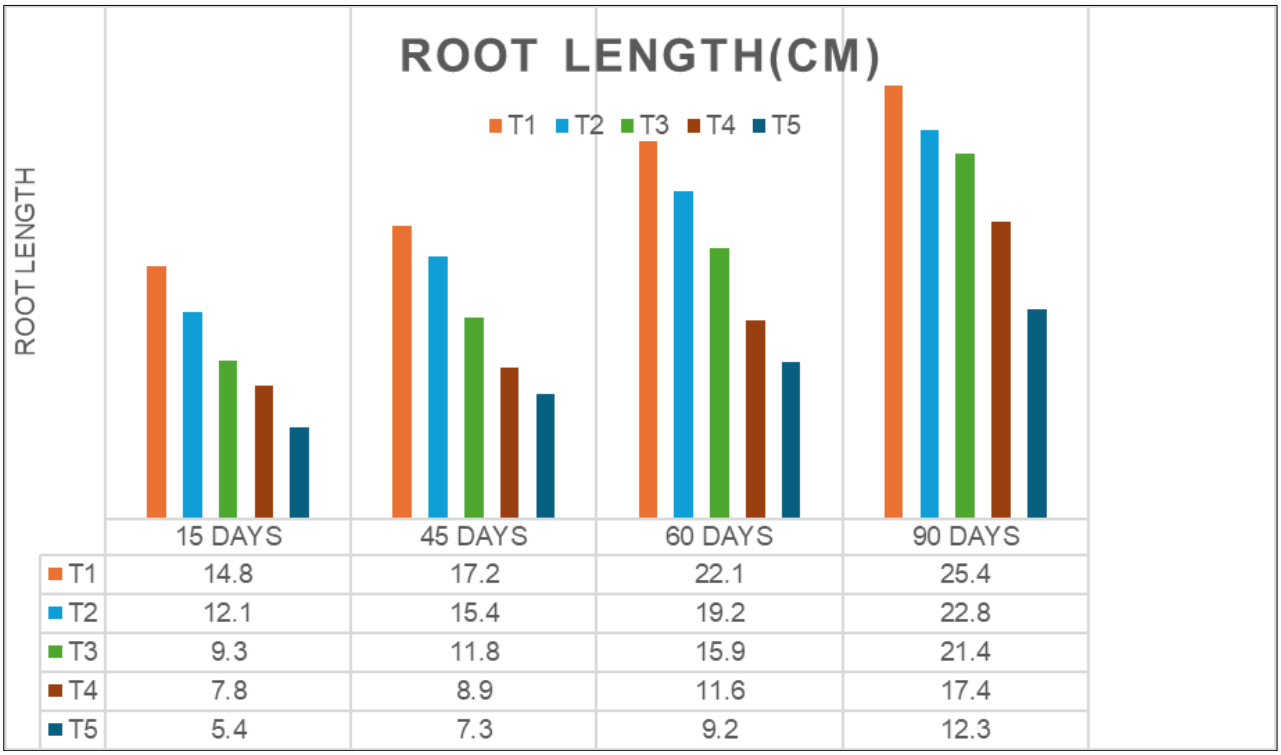
Table 4: Effect of bio agents on the growth of *Sclerotium rolfsii*

Microbial strain	Radial growth (cm)	Inhibition Percentage (%)
P. fluorescens	1.32 ± 0.1^{b}	84.82 ± 1.66^{c}
T. harzianum	0.45 ± 0.02^{c}	96.88 ± 0.2^{b}
B. subtilis	1.6 ± 0.15^{a}	87.18 ± 1.69^{d}
Control	9.2 ± 0^{d}	0.0 ± 0^{a}

Effect of various treatments on growth parameters of tomato crop (Year 2023-24) Root length

After 15 days of transplanting, the effects of the various microorganisms used to fortify the vermicompost were clearly seen on the growth characteristics. When compared

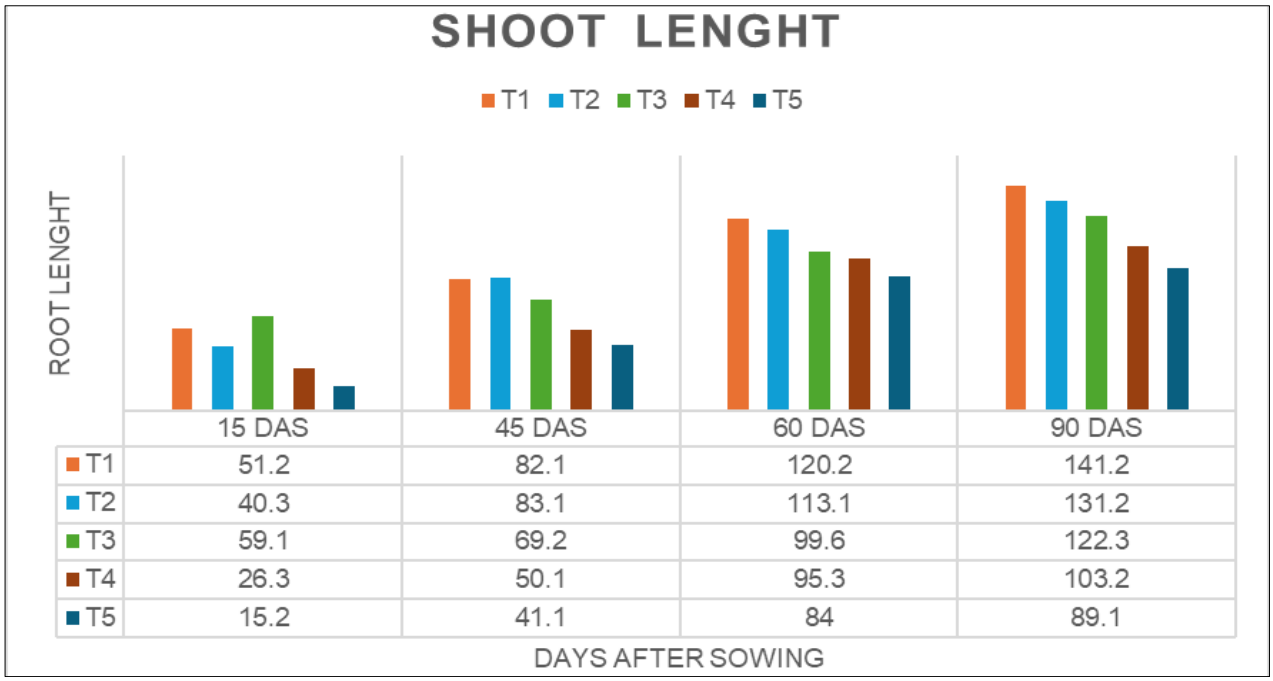
to the control, all treated plants had noticeably longer roots. After 15 days of sowing, tomato plants treated with vermicompost enhanced with Trichoderma had the longest roots (14.8 cm), followed by T-2 (12.1 cm) and T-3 (9.3cm). Following a seeding, similar patterns were seen 45, 60, and 90 days later (Figure 1).



	15 DAYS	45 DAYS	60 DAYS	90 DAYS
T1	14.8	17.2	22.1	25.4
T2	12.1	15.4	19.2	22.8
T3	9.3	11.8	15.9	21.4
T4	7.8	8.9	11.6	17.4
T5	5.4	7.3	9.2	12.3

Fig 1: Effect of different treatments on root length of tomato

Shoot length: Shoot length was recorded at 15, 45, 60 and 90 days after sowing. At each interval, tomato plants treated with vermicompost enhanced with Trichoderma exhibited the largest shoot length. Maximum shoot length 51.2cm was observed in T-1 followed by T-2 (40.2cm) and T-3 (59.1cm) after 15 DAS. Similar pattern was observed after 45, 60 and 90 days after sowing. After 90 DAS maximum shoot length was observed in T-1 (141.25 cm). After 90 DAS significant difference was observed in treated and control plants (Figure 2).

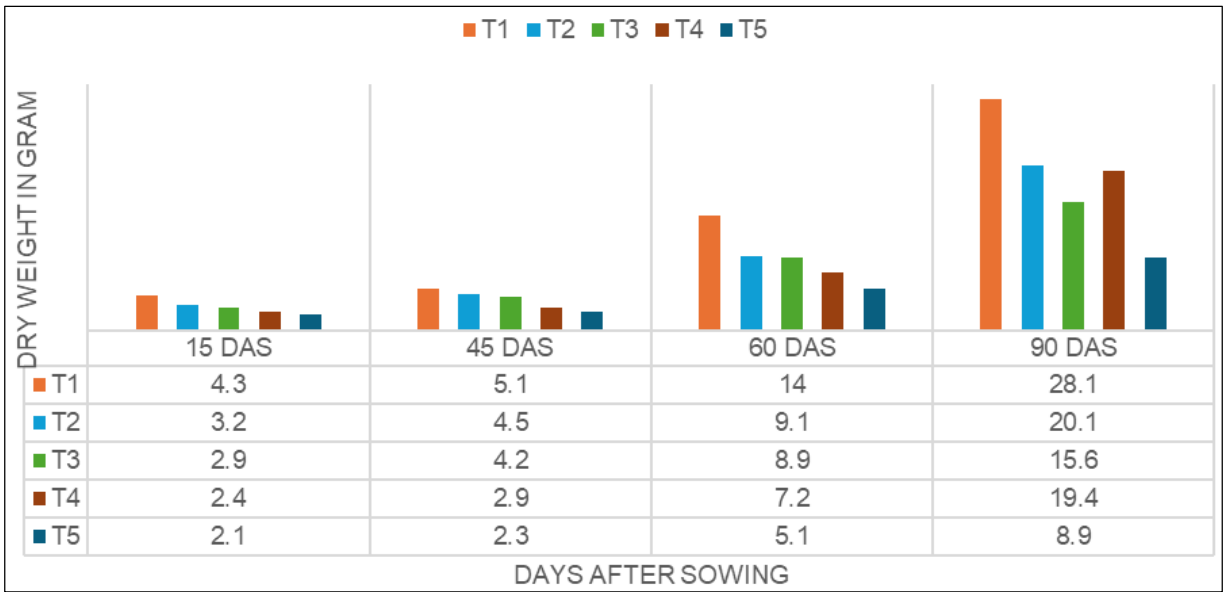


	15 DAS	45 DAS	60 DAS	90 DAS
T1	51.2	82.1	120.2	141.2
T2	40.3	83.1	113.1	131.2
T3	59.1	69.2	99.6	122.3
T4	26.3	50.1	95.3	103.2
T5	15.2	41.1	84	89.1

Fig 2: Effect of different treatments on shoot length of tomato

Dry weight

After sowing, dry weight was observed at 15,45,60,90 days. Maximum dry weight was observed in plants treated with vermicompost fortified with *Trichoderma*. After 15DAS, 4.3 g dry weight was in T-1 followed by T-2 and T_3 (Figure 3).



	15 DAS	45 DAS	60 DAS	90 DAS
T1	4.3	5.1	14	28.1
T2	3.2	4.5	9.1	20.1
T3	2.9	4.2	8.9	15.6
T4	2.4	2.9	7.2	19.4
T5	2.1	2.3	5.1	8.9

Fig 3: Dry Weight in gram

Effect of biofortified vermicompost on defense related enzymes in tomato plants challenged with *S. rolfsii*

Phenylalanine Ammonia Lyase (PAL)

All therapies showed a considerable increase in PAL levels up to 48 hours, after which its activity decreased. The plant planted in vermicompost fortified with T. harzianum (T_1) showed the highest PAL activity in its leaves at 48 hours, followed by T_2, T_3, and T_4. T_1 revealed 2.8-fold increases in PAL activity at 48 hours compared to the control. Comparing T_2 and T_3 to the control, they simultaneously displayed 3.and 2.5 fold increases in PAL accumulation. Additionally, compared to the control, plants from non-fortified vermicompost exhibited increased PAL accumulation. When compared to the control, the plant only with vermicompost (T_4) at 48 hours demonstrated a 2.4-fold increase in PAL accumulation (Figure 4)

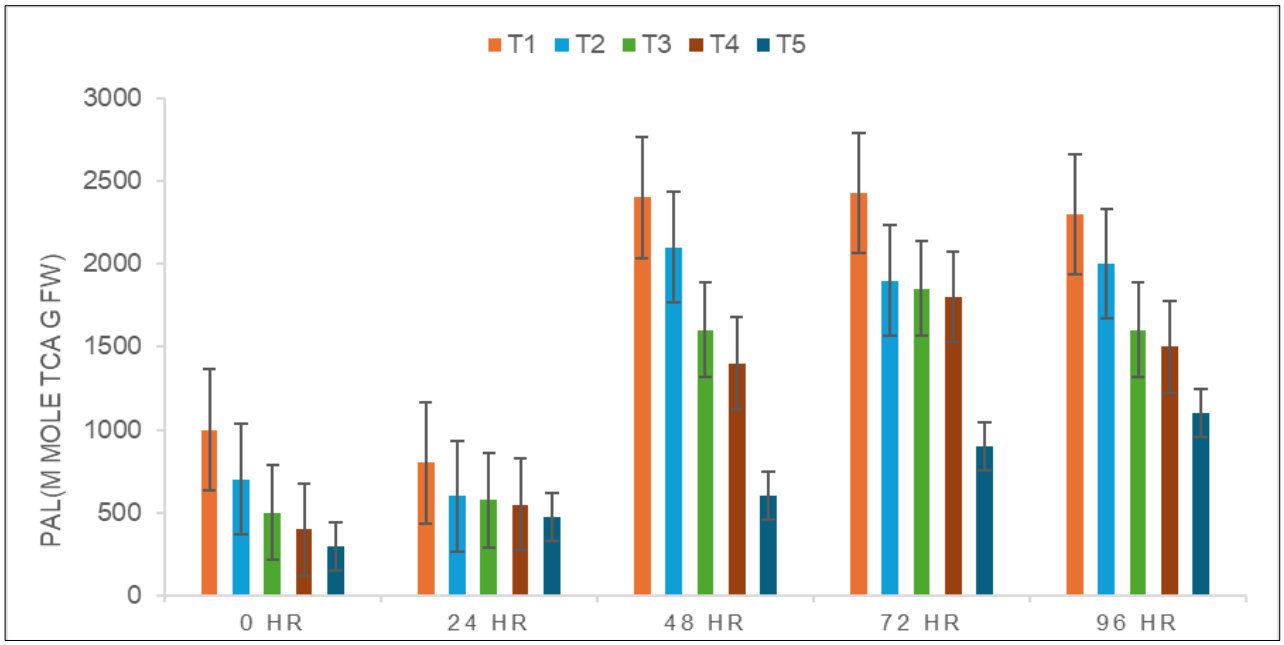


Fig 4: Hours after pathogen challenge

Figure 5 PAL activity in tomatoes grown from seeds planted in soil supplemented with Biofortified vermicompost and challenged with Sclerotium rolfsii at various time intervals The means of three replicates are used to express the results, and the standard deviation of the mean is shown by vertical bars. According to Duncan's multiple range test at $p<0.05$, different letters indicate significant differences among treatment results collected at the same time interval.

Peroxidase (PO): PO levels dramatically rose in all treatments for the first 72 hours, after which its action decreased. The plant planted in vermicompost fortified with T. harzianum (T_1) showed the highest PO activity in its leaves at 72 hours, followed by T_2, T_3, and T_4. T_1 displayed an 8.4 times increase in PO build-up after 72 hours compared to the control. In contrast to the control, T_2 and T_3 simultaneously displayed 8 and 5.5 fold increases in PO activity. Additionally, plants that received solely vermicompost accumulated more PO than the control. Comparing plant alone with vermicompost (T_4) to control, the accumulation of PO increased 3.21 and 2.4 times at 48 and 72 hours, respectively (Figure 5).

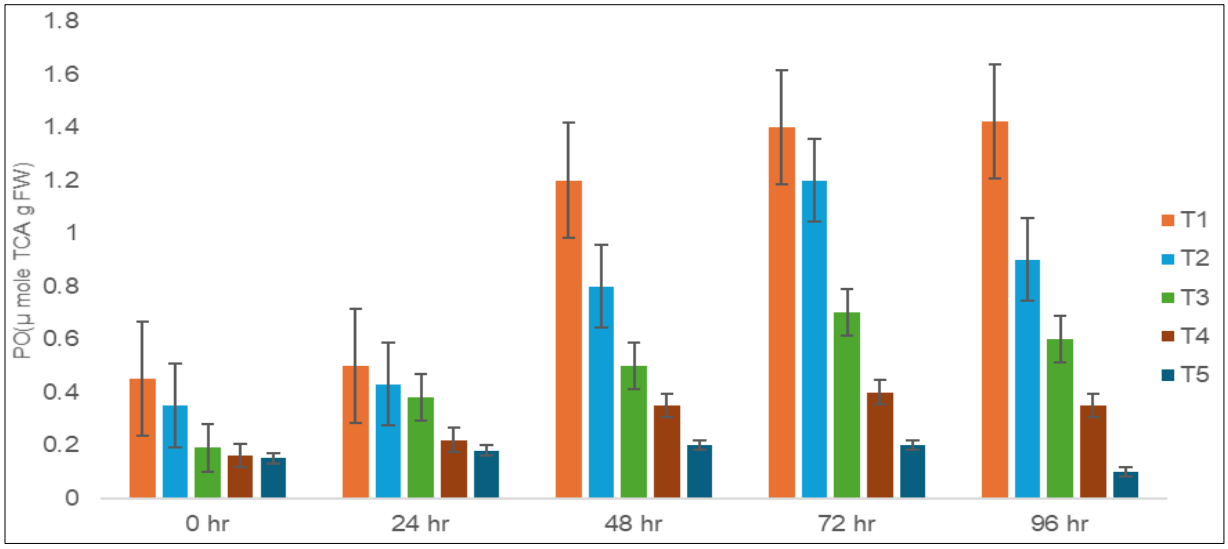


Fig 5: PAL activity in tomatoes grown

Figure 5 PO activity in relation to microbially fortified vermicompost. The means of three replicates were used to express the results, and the standard deviation of the means is shown by vertical bars. According to Duncan's multiple range test at $p \leq 0.05$, different letters indicate significant variations among treatments of results taken at the same time frame.

Polyphenol oxidase (PPO): PPO activity decreased after its levels dramatically rose in all treatments for up to 72 hours. The plant planted in vermicompost fortified with T. harzianum (T_1) showed the highest PO activity in its leaves at 72 hours, followed by T_2, T_3, and T_4. T_1 revealed a 6-fold increase in PPO build-ups at 72 hours compared to the control. When compared to the control, the plant only with vermicompost (T_4) at 72 hours demonstrated 2.5 times higher PPO accumulation (Figure 5).

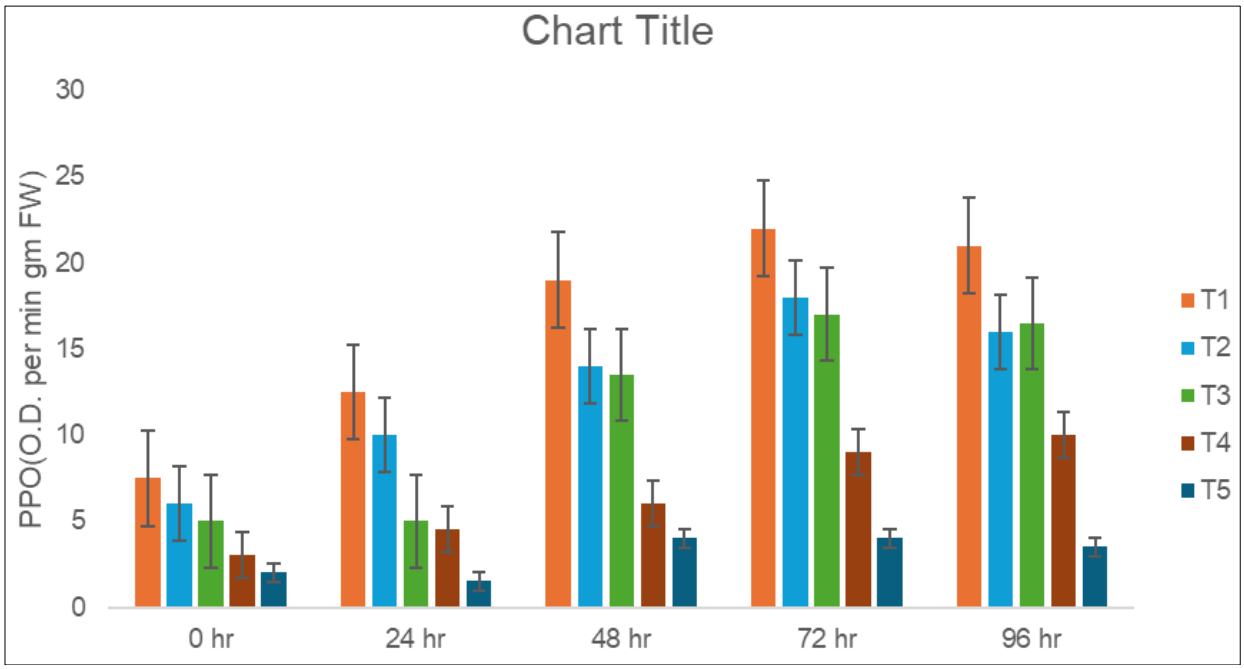


Fig 6: PPO activity in relation to microbially fortified vermicompost

Figure 6 PPO activity in relation to microbially fortified vermicompost. According to Duncan's multiple range test at $p \leq 0.05$, different letters indicate significant variations among treatments of results taken at the same time frame.

Total phenol content (TPC) in tomato: The TPC's activity sharply declined after a maximum increment at 48 hours in T_1, following the same pattern as the PAL. There is a noticeable difference in the TPC content between the various treatments. In T_1, the maximum phenolic content was noted 48 hours later. At 48 hours, the total phenolic content in T_1, T_2, T_3, and T_4 was higher than in the control by 7, 6.4, 4.9, and 3.7 fold, respectively (Figure 6).

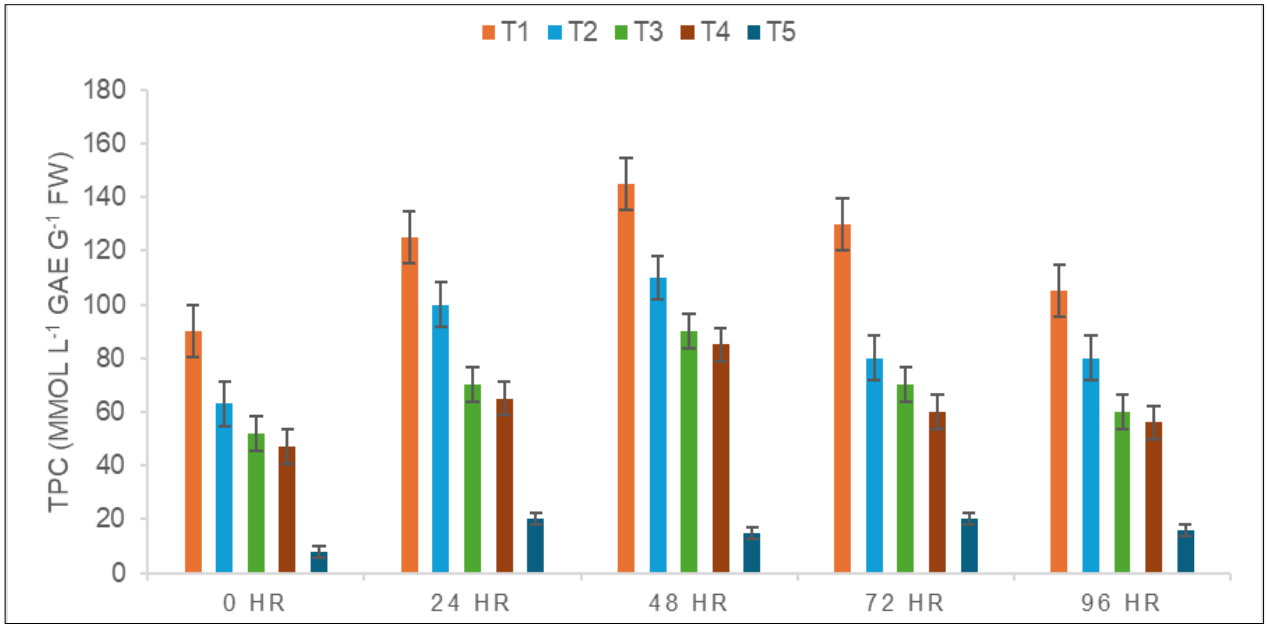


Fig 7: TPC activity in tomatoes under various treatments at various time periods

Figure 7 TPC activity in tomatoes under various treatments at various time periods. The means of three replicates are used to express the results, and the standard deviation of the mean is shown by vertical bars. According to Duncan's multiple range test at $p < 0.05$, different letters indicate a significant difference among treatment results collected at the same time interval.

Discussion

Effect of microbial fortified vermicompost on growth parameters of tomato crop;

It is generally acknowledged that, as compared to synthetic fertilizers, the application of composts and vermicompost as soil amendments can enhance soil nutrient status, boost soil health, and improve most crop plant attributes. The notable outcomes in terms of increased yield and decreased disease incidence were attained with the introduction of biofortified vermicompost. The study's findings demonstrated a discernible difference between tomato plants grown in vermicompost alone and in vermicompost enhanced by microorganisms in terms of growth promotion. There were very noticeable differences between the treatments in terms of dry weight, shoot length, and root length. The new study's findings are consistent with the previous report.

The current study's findings are consistent with a report by Wang *et al*., which said that applying vermicompost to tomatoes in addition to bio inoculants encouraged development. In their investigation, they tried a variety of treatments and discovered that the best results were gained when they combined treatments, such as vermicompost, Bacillus subtilis, Tricoderma, and the mycorrhizal fungus Glomus mossieae. Similar findings were also reported by Bachman and Metzer, who began using vermicompost and biopesticides in brinjal to improve productivity and manage worms. The results of this investigation are also consistent with experimental results that demonstrated the critical function that vermicompost-or its combination with a biopesticide based on Pseudomonas fluorescens-has played in promoting growth in tomato plants.

Effect of biofortified vermicompost on activity of defences related enzymes in tomato;

Plants have a variety of defense mechanisms to fend off invaders. Biofortified vermicompost can strengthen these defences and speed up the cellular defense response, which is the most cost-effective option, but it only becomes apparent when a plant is confronted with a pathogen. The current study's findings showed that when Sclerotium rolfsii was present, tomato plants treated with biofortified vermicompost showed increased activity levels of defense-related enzymes and accumulated phenols in their leaves. ISR was methodically created in response to beneficial microorganisms from vermicompost and specific biocontrol agents colonizing plant roots. An early oxidative burst and a greater upregulation of defense genes were also components of this cellular response. The current study's findings regarding the induction of defense proteins and enzymes can be explained as a defense reaction in tomatoes caused by pathogen invasion. The outcomes showed that biofortified vermicompost treatment resulted in a multiplication of defense-related enzyme activity, including PAL, PO, PPO, SOD, and phenols, indicating their function in disease resistance. The plant treated with vermicompost fortified with T. Harzianum exhibited the highest levels of enzyme activity.

Phenols have a variety of roles in plant defences, including the creation of the signaling chemical salicylic acid, the strengthening of cell walls, and antibacterial activity. The highest levels of PAL activity were observed in leaves from plant growth in vermicompost fortified with T. Harzianum (T_1) at 48 hours. The results align with the research on how Bacillus subtilis and vermicompost control the damping-off illness in psyllium. Amooaghaie & Co. It has been reported that Bacillus subtilis and vermicompost both cause systemic resistance by signaling with nitric oxide. Together, the applications caused beta-1,3-glucanase, PAL, PPO, and other defense-related enzymes to accumulate. They also successfully decreased lipid peroxidation in psyllium leaves.

A higher degree of host plant resistance to pathogen is attained as a direct result of the increased antimicrobial activity, which is somewhat correlated with the increased PAL activity. Together with other ascorbate-glutathione cycle enzymes, the enzymes SOD and PO aid in the scavenging of free radicals. SOD belongs to the class of

antioxidative enzymes that catalyze the conversion of O2 to H2O2 and O2, two processes that are critical for reducing the harm that oxidative stress causes. PO enzymes are involved in a wide range of physiological processes, including as lignification, auxin metabolism, cross-linking of cell wall proteins, and defense against phytopathogens. They catalyse the reduction of H2O2 by transporting electrons to different donor molecules (Sarma *et al*., 2015).

Analysis of the plant following pathogen infection revealed that PO levels dramatically rose in all treatments for up to 72 hours, after which its activity decreased. At 72 hours, the highest PO activity was seen in the leaves of plants growing in vermicompost that had been fortified with Trichoderma harzianum (T_1). Vermicompost bio fertilizer was shown to produce the highest levels of PO and SOD activity in green house cucumbers following a 72-hour Pythium aphanidermatum challenge, according to a prior study with similar findings. Increased PO activity in treated plants after receiving biofortified vermicompost treatment has been shown to potentially result in lignin build up, which is a crucial physical barrier to prevent pathogen invasion. Increased plant tissue PPO activity against pest insects and phytopathogens has been documented in a number of advantageous plant microbes interaction. Increased antimicrobial action and a reduction in the build-up of harmful oxidation products are somewhat correlated with increased PAL and PPO activity, which results in improved pathogen resistance. The leaves of the plants cultivated in the vermicompost supplemented with Trichoderma harzianum for 72 hours showed the highest PO activity in the current investigation. An additional sign of enhanced pathogen tolerance is the increased PPO activity in every plant treated with biofortified vermicompost. PAL, PO, and PPO defence-related enzyme levels were elevated in plants treated with Bacillus subtilis and vermicompost under stress caused by Sclerotium rolfsii. Psyllium in plantago. Our findings also concur with those, who found that treating tomatoes with biofortified vermicompost against Sclerotium rolfsii (basal stem rot) improved the activity of PAL, PO, and PPO.

A partial explanation for the observed delay in the development of symptoms may be found in the increased activity of defence-related enzymes in the plant treated with biofortified vermicompost. This response of the host cells to the pathogen is used to fully inhibit the fungus's growth without causing additional damage to the surrounding tissue.

Conclusion

The three biocontrol agents-Pseudomonas fluorescens, Bacillus subtilis, and Trichoderma harzianum-were utilized to fortify the vermicompost separately after being found to be compatible and capable of reducing soil-borne illnesses in a variety of crops. The effects of biofortified vermicompost on disease prevention and plant growth were observed, and all treated plants had noticeably longer roots than the control group. Biological Plants from each treatment were analysed to determine how the biofortified vermicompost affected the enzymes linked to defence. Up to 48 hours, all treatments showed a considerable increase in PAL, PPO, and PPO levels. This was followed by a decrease in its activity. In leaves from plants grown in vermicompost fortified with Trichoderma harzianum (T_1), the highest levels of PAL, PO, and PPO activity were observed. The biological management of a tomato fungus disease caused by Sclerotium rolfsii proved to be highly effective when vermicompost was enriched with a particular biocontrol agent. Enzymes linked to defence were noticeably elevated in tomatoes cultivated in soil mixed with strengthened vermicompost. The fortified vermicompost had a significant effect on different growth indices and plant morphology. The beneficial microorganisms that vermicompost hosts may be the cause of its reported growth improvement and biocontrol capability.

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